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Importance of ultrafilterable plasma factors in maintaining tubular reabsorption

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Importance of ultrafilterable plasma factors in maintaining tubular reabsorption. The recollection technique was used to measure reabsorptive rates in rat proximal tubules during *in situ* microperfusion with an artificial perfusate or an ultrafiltrate of rat plasma. During one minute of microperfusion reabsorptive rates were no different with the two solutions. Reabsorption decreased 34% when remeasured at ten minutes in the same tubule during continuous perfusion with the artificial solution. No decrease occurred when paired measurements were made at one and ten minutes during continuous perfusion with an ultrafiltrate of plasma. These results suggest that some constituents of plasma other than major electrolytes and glucose are necessary within the lumen to maintain a constant rate of proximal tubular reabsorption. The effect of perfusion rate on the rate of reabsorption was reinvestigated by the recollection technique using plasma ultrafiltrate as the perfusate. Overall, a two-fold change in perfusion rate did not change reabsorptive rate. When only long tubule segments were analyzed (increasing the accuracy of measuring changes) a relationship between perfusion rate and reabsorptive rate was apparent. The change in reabsorptive rate averaged 40% of the change in perfusion rate. The results of these studies indicate that unidentified constituents of normal glomerular filtrate as well as intraluminal flow rate can influence proximal tubular reabsorption.

Importances des facteurs plasmatiques ultrafiltrables dans le maintien de la réabsorption tubulaire. La technique des recollections a été utilisée pour mesurer le débit de réabsorption de tubes proximaux de rat durant la perfusion in situ par un perfusat artificiel ou un ultrafiltrat de plasma de rat. Au cours de microperfuions d'une minute les débits de réabsorption n'étaient pas différents avec les deux solutions. La réabsorption était diminuée de 34% quand elle a été mesurée à la 10e minute dans le même tubule au cours d'une perfusion continue avec la solution artificielle. Aucune diminution n'a été observée au cours de déterminations comparatives après une et 10 minutes de perfusion continue d'un ultrafiltrat de plasma. Ces résultats suggèrent que certains constituants du plasma autres que les électrolytes majeurs et le glucose sont nécessaires, à l'intérieur de la lumière, pour le maintien d'un dèbit constant de réabsorption proximale. L'effet du débit de perfusion sur le débit de réabsorption a été étudié par la technique de recollection et en utilisant de l'ultrafiltrat de plasma comme perfusat. Dans l'ensemble, une modification d'un facteur deux du débit de perfusion n'a pas modifié le débit de réabsorption. Quand seuls des longs tubules ont été étudiés (ce qui augmente la précision des déterminations) une relation entre les débits de perfusion et de réabsorption était en moyenne 40% de celle du débit de perfusion. Ces résultats indiquent que des constituants non identifiés du filtrat glomérulaire ainsi que le débit de liquide intraluminal peuvent influencer la réabsorption tubulaire proximale.

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Microperfusion of tubular segments *in vivo* has been used in several laboratories to study factors that may influence reabsorption [1–7]. Artificial perfusates have been employed and, thus, the findings may not be applicable to physiological circumstances when the tubular fluid is glomerular filtrate. Burg and Orloff reported that reabsorptive rates by the isolated proximal tubule perfused *in vitro* with an artificial perfusate were 50% lower than when tubules were perfused with an ultrafiltrate of plasma [8]. Moreover, it has been demonstrated that sugars, amino acids and other organic acids are capable of enhancing transepithelial transport of sodium or the facilitated diffusion of sodium across cell membranes [9]. These and other substances normally present in the glomerular filtrate could play a role in the normal process of tubular sodium transport, and their absence in artificial perfusates could affect the experimental results obtained when renal tubular segments are perfused *in vivo*.

For these reasons it seemed important to compare reabsorptive rates by proximal tubular segments perfused with an artificial solution to reabsorptive rates measured when the perfusate was an ultrafiltrate of normal plasma. In the present study it was found that artificial perfusion solution, but not an ultrafiltrate of plasma, was associated with a decline in the rate of reabsorption during continuous *in vivo* perfusion of proximal tubular segments. Therefore, we have reinvestigated the influence of tubular fluid flow rate (or load) on the rate of proximal tubular reabsorption.

Methods

Experiments were performed in 84 male Sprague-Dawley rats weighing between 190 and 340 g that were prepared for micropuncture as described previously [7]. Animals received a maintenance infusion of a modified Ringer's solution (Na 142, K 4.0, Cl 116 and HCO_3 30, mEq/liter) at 50 µl/min/kg body weight which contained inulin to achieve concentrations in plasma between 50 and 100 mg/100 ml and PAH in an amount appropriate for clearance measurements. Microperfusion of tubular segments was performed with a constant speed pump that has been described in detail previously [4]. A thin-tipped pipette (O.D. 6 µ) was inserted into a superficial convolution and a bolus of either 1% lissamine green or mineral oil was injected for the purpose of mapping sequential proximal convolutions and the distal tubule of the same nephron. This injection pipette was withdrawn, and approximately five minutes later the perfusion pipette was inserted into one of the early proximal convolutions. The perfusion system had been operating for approximately 30 minutes and was continued uninterrupted during puncture of the tubule and throughout the remainder of the experiment. A castor oil block was then injected proximal to the perfusion pipette, and a tear was made proximal to this oil block to permit the escape of glomerular filtrate. A collecting pipette was then inserted downstream in the proximal tubule or in the early distal tubule, and an oil block of five to seven tubular diameters in length was injected from the collecting pipette, after which aspiration of the perfused fluid was begun immediately. The rate of aspiration was adjusted so as to maintain a stable position of both the proximal and distal oil blocks. Samples of perfused fluid of 15 to 80 nl were collected over a period of one to seven minutes.

Total collections of the perfused fluid from distal tubules were performed by a modified technique that involved sealing the puncture site with the oil block and a long-tipped collection pipette with an O.D. of 7 to 8 µ. This method for collecting distal tubular fluid has been found more reliable than previously employed methods, which are frequently associated with incomplete collections [10]. When collections were terminated, the tip of the collecting pipette was sealed with mineral oil from the surface of the kidney. Samples were transferred into a calibrated glass capillary for measuring volume. A portion of the sample was transferred under oil with a calibrated pipette for measuring radioactivity. The same calibrated pipette was used for measuring radioactivity in the perfusion solution.

The perfusion rate (*PR*) *in vivo* was calculated for each tubular perfusion as follows:

$$PR = \frac{CF \cdot V}{PF}$$

where V is rate of downstream collection of perfusate and CF and PF are the concentrations of the volume marker in collected fluid and perfused fluid, respectively. At the completion of each experiment the perfusion rate was measured *in vitro* also by making a timed collection into a calibrated glass capillary at the same pump settings used during the experiment. The rates of perfusion were varied intentionally among tubules and in some experiments the same tubule was perfused at two different rates.

The artificial perfusate contained Na 142, K 4.7, Ca 2.0, Cl 118, HCO_3 30, glucose 5 and urea 2.2 mmoles/liter, 100 mg/100 ml lissamine green and either ^{125}I-iothalamate or inulin-methoxy-^{3}H as the marker for volume change. The ultrafiltrate of plasma was prepared by filtering fresh rat plasma through a Diaflo UM-10 membrane on an Aminco ultrafiltration apparatus. Lissamine green and inulin-methoxy-^{3}H were added to the ultrafiltrate, and NaCl and $NaHCO_3$ were added to achieve a concentration of these ions approximately equal to that of the artificial perfusate. The plasma ultrafiltrate was prepared fresh each week. The average composition of the ultrafiltrate was Na 144 ± 5, K 4.4 ± 0.5 mEq/liter. The paired average composition of the plasma of the same animals was Na 141 ± 7, K 4.6 ± 1.5 mEq/liter.

In nine animals collections of perfused fluid were begun within one minute; ten minutes after the first collection was completed, a re-collection of approximately the same volume was begun while the tubule was being perfused at a constant rate throughout with the artificial perfusate. Forty of these paired collections were from late proximal tubules and three were from early distal tubules. Proximal collections lasted up to three minutes; distal collections lasted up to seven minutes. In 31 additional animals only the first collection was taken. In 20 animals collections and re-collections were made at one and ten minutes during continuous perfusion at the same rate with the ultrafiltrate of plasma. In these experiments 48 paired collections were made from late proximal tubules and 17 paired collections were from early distal tubules. In another 67 tubules perfused with the ultrafiltrate only one collection was made.

In 21 animals 65 tubules were perfused with the ultrafiltrate, and after the first collection the perfusion rate was either increased or decreased in random fashion. Five to ten minutes after the rate of perfusion was changed a second collection was made at the site of the initial collection. Fifty paired collections were made from late proximal tubules and 21 from early distal tubules.

In 17 experiments in which ^{125}I-iothalamate was the marker for volume change, the collected fluid was analyzed for chemical inulin as a means of checking the collection for contamination with interstitial fluid or fluid from adjacent tubules. Two of these samples were discarded because they contained detectable inulin. One hundred fifty-four tubules were injected with latex, and the kidney was macerated in 6 N HCl for microdissecting the perfused tubular segments. The length of the perfused segment was measured from the latex cast in order to calculate the reabsorptive rate per unit length of tubule. The total rate of reabsorption (C) from the site of perfusion to the site

of collection was calculated as $C=PR-V$, and in tubules in which the length of perfused segment was measured this quantity could be expressed as nl/min/mm. Otherwise C is expressed as nl/min. In some tubules in which total collections were not made, C was calculated from the average *in vivo* perfusion rate measured in adjacent tubules at the same pump setting.

^{125}I was measured in a well-type gamma spectrometer (Packard Instruments Co., Downers Grove, Ill., and ^{3}H was measured in Bray's solution [11] using a Nuclear of Chicago liquid scintillation spectrometer. Chemical inulin in collected perfusate was determined by a method previously described [7]. The significance of differences between first and second collections was determined by Students "t" test for paired data.

Results

Perfusion rates and recovery of volume markers. The perfusion rate *in vivo* was calculated by collecting all tubular fluid downstream in either the late proximal tubule or the early distal tubule. In each experiment the *in vivo* measurements were averaged and compared to an *in vitro* measurement of perfusion rate at the same pump speed made by collecting the perfusion fluid in a calibrated glass capillary. The results of these comparisons are shown in Fig. 1. Recovery of the perfused markers *in vivo* based on delivery of the pump *in vitro* averaged 95.6±8.9% ($N=40$) for iothalamate and 95.2±12.2% ($N=29$) for inulin. Overall, there was no apparent difference in recoveries of the markers related to the site of collection or whether the perfusate was the artificial solution or an ultrafiltrate of plasma. In nine experiments with artificial perfusate and 20 experiments in which the perfusate was an ultrafiltrate of plasma the *in vivo* perfusion rate was determined during the first one minute of perfusion and again after ten minutes of perfusion by re-collection from the same tubular site. These data are shown in Fig. 2. Recovery of the perfusion marker at ten minutes averaged 101.4% of that at one minute for the artifical perfusate and 101.2% when the perfusate was an ultrafiltrate of plasma. Thus, overall recoveries of iothalamate and inulin were close to 100% and were uninfluenced by the site of collection (proximal or distal tubule), the nature of the perfusate (artificial or plasma ultrafiltrate) or re-collection after ten minutes of perfusion.

Effect of duration of perfusion on reabsorptive rate. In 43 tubules of nine animals early proximal tubules were perfused at 12.2 to 22.5 nl/min with the artificial perfusate, and initial collections were made during the first one minute of perfusion either downstream in the proximal tubule or in the early distal tubule. After this initial collection the perfusion was continued uninterrupted and at ten minutes re-collections were made from the original tubular sites. Comparisons of the reabsorptive rates at one and ten minutes using the artificial perfusate are shown

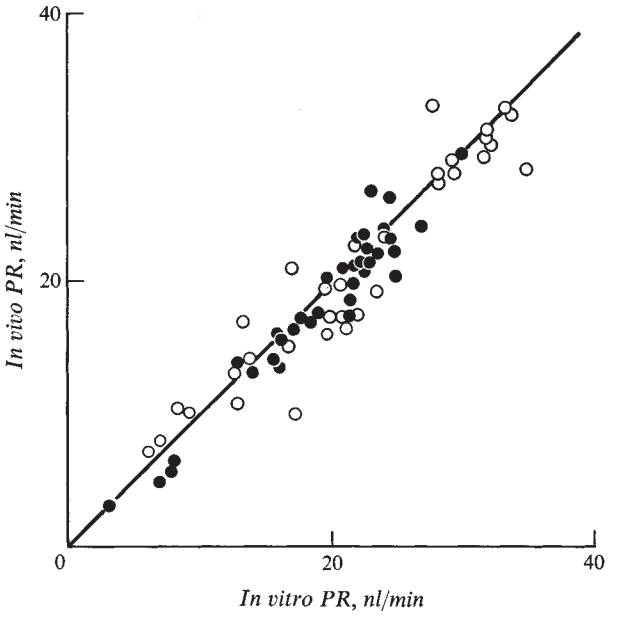

Fig. 1. *Comparison of perfusion rates* (PR) *measured* in vitro *and at the same pump setting* in vivo. Solid symbols are data obtained with ^{125}I-iothalamate as the volume marker, and open symbols are data with inulin-methoxy-^{3}H. The diagonal line represents identical values. Average recoveries of ^{125}I-iothalamate were 95.6±8.9%, $N=40$, of inulin-methoxy-^{3}H 95.2±12.2%, $N=29$.

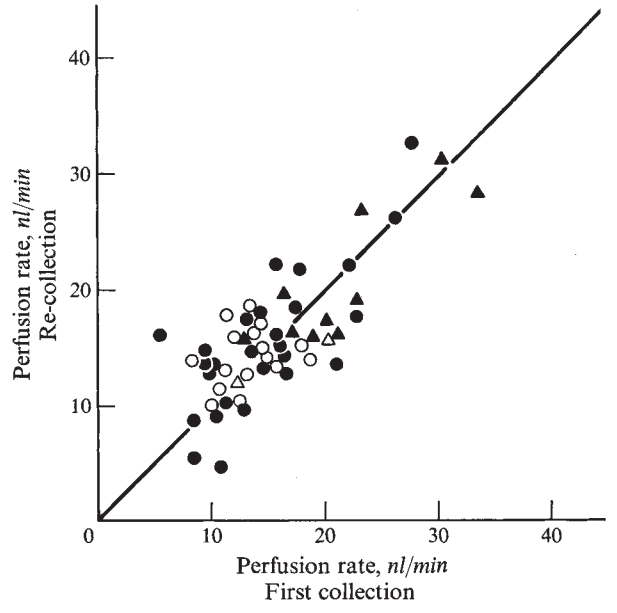

Fig. 2. *Comparison of perfusion rates measured* in vivo *during the first collection and during re-collection after ten minutes of continuous perfusion at the same rate.* Open symbols represent data obtained by perfusion with artificial perfusate and solid symbols data during perfusion with an ultrafiltrate of plasma. Circles are collections from proximal tubules and triangles collections from distal tubules. The diagonal line represents no change.

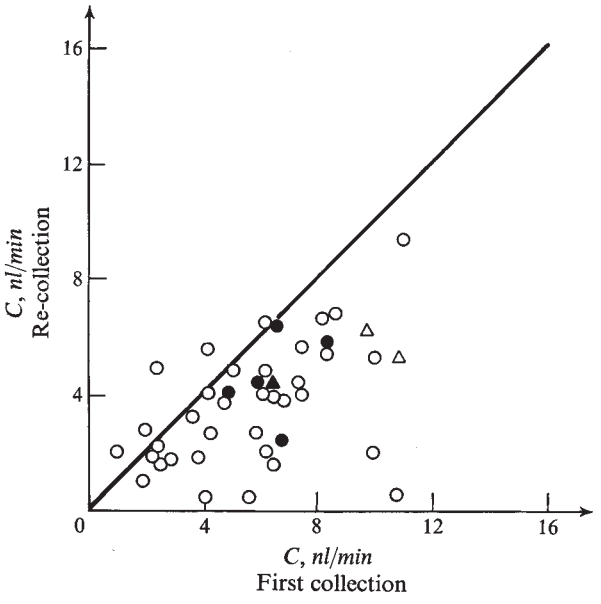

Fig. 3. *Effect of continuous microperfusion with artificial perfusate on reabsorptive rate* (C) *at the site of collection.* The first collection was begun within one minute of perfusion and the re-collection ten minutes after the completion of the first collection from the same site. Circles represent collection pairs from late proximal tubules; triangles represent collection pairs from distal tubules. Solid symbols represent collection pairs in which recoveries of the perfusion marker were identical.

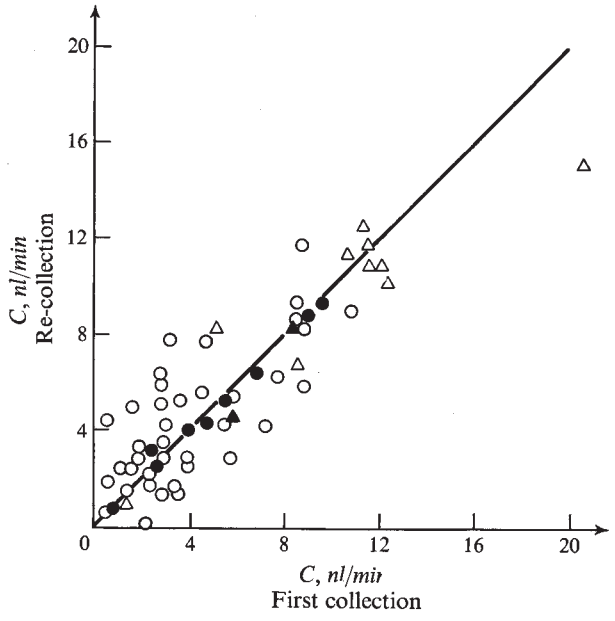

Fig. 4. *Effect of continuous microperfusion with an ultrafiltrate of plasma on reabsorptive rate* (C) *at the site of collection.* Representation of data is the same as in Fig. 3.

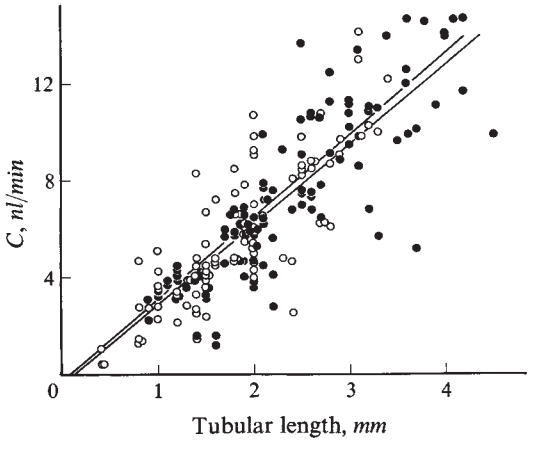

Fig. 5. *Reabsorptive rate* (C) *during the first one minute of microperfusion with artificial perfusate (solid symbols) and an ultrafiltrate of plasma (open symbols).* The length of tubular segment perfused was measured from microdissected latex casts. For both perfusion solutions the rate of reabsorption was linearly correlated with segment length. For the artificial perfusate, $C=-0.51+3.34$ length, $r=0.81$, $N=110$. For the ultrafiltrate of plasma, $C=-0.49+3.44$ length, $r=0.80$, $N=88$. The two regression lines are traced.

in Fig. 3. In most instances reabsorption was lower at ten minutes than at one minute of perfusion. Reabsorption at the site of collection averaged 5.6 ± 2.7 nl/min at one minute and 3.7 ± 2.0 nl/min at ten minutes ($P<0.01$). It is evident from the data of Fig. 2 that this measured decrease in reabsorptive rate at ten minutes was not due to a systematic decrease in recovery of the volume marker.

In 65 tubules of 20 animals, collections and re-collections were made at one and ten minutes, respectively, either from the late proximal or early distal tubule during continuous perfusion of the tubule with an ultrafiltrate of plasma. Comparison of these reabsorptive rates at one and ten minutes in the same nephron are shown in Fig. 4. The rate of reabsorption up to the sites of collection averaged 5.3 ± 3.9 nl/min at one minute and 5.5 ± 3.5 nl/min at ten minutes. These values were not statistically different ($P>0.60$). Thus, the fall in reabsorptive rate that occurred after ten minutes of perfusion with the artificial perfusate (Fig. 3) was not evident when the tubule was perfused with an ultrafiltrate of plasma.

During the first one minute of perfusion the overall mean rate of reabsorption of the artificial perfusate was not significantly different from that observed when tubules were perfused with the ultrafiltrate of plasma. Measurements of tubular length between the site of perfusion and the site of collection were made in 110 proximal tubules perfused with artificial perfusate and in 44 proximal tubules perfused with the ultrafiltrate of plasma. The relationship between reabsorptive rate and segment length for these tubules is shown in Fig. 5. During the first one minute of perfusion reabsorption with the artificial perfusate averaged 3.1 ± 0.8 nl/min/mm and 3.1 ± 1.0 nl/min/mm with the ultrafiltrate of plasma.

Effect of perfusion rate on rate of reabsorption. Sixty-five tubules of 21 animals were perfused with plasma ultra-

Table 1. Relationship between perfusion rate and tubular reabsorption during microperfusion with ultrafiltrate of plasma

	Sampling site	Perfusion rate *nl/min*	*CF/PF*	*C* *nl/min*
1.	P	21.2	1.26	4.4
		12.8	1.62	4.9
2.	P	16.1	2.19	8.7
		26.1	1.51	8.8
	P	10.1	1.04	0.4
		24.5	1.02	0.5
	P	19.3	1.25	3.9
		27.7	1.16	3.8
	P	33.1	1.12	3.5
		17.9	1.18	2.7
3.	P	16.3	1.60	6.1
		33.2[a]	1.19	5.3[a]
	P	21.9	1.14	2.7
		32.7	1.01	0.3
4.	D	22.5[a]	2.46	13.4[a]
		32.9[a]	1.95	16.0[a]
	D	22.5	2.37	13.0
		32.9[a]	1.52	11.3[a]
5.	D	14.5	2.34	8.3
		21.7	2.55	13.2
	D	23.3	1.41	6.8
		30.2	1.23	5.6
	D	32.5	1.24	6.3
		16.5	2.07	8.5
	D	19.5	1.74	8.3
		37.7	1.41	11.0
	D	20.2	2.32	11.5
		43.0	1.68	17.4
	D	11.9	1.01	0.1
		8.8	1.01	0.1
		23.7	1.07	1.6
6.	D	36.6	1.29	8.2
		15.1	1.26	3.1
	P	15.8	1.08	1.2
		31.8	1.01	0.3
		18.2	1.13	2.1
7.	P	28.7[a]	1.20	4.8[a]
		15.1	1.42	4.5
	P	15.7	1.43	4.7
		9.7	1.16	1.3
	P	39.3	1.20	6.6
		19.2	1.31	4.6
	P	25.1	1.38	6.9
		12.5	1.41	3.6
8.	P	19.6	1.66	7.8
		25.4	1.32	6.2
		11.8	1.86	5.5
	P	12.7	1.74	5.4
		33.8	1.14	4.2
	D	11.0	1.99	5.5
		8.0	3.54	5.7
	D	25.1	2.15	13.4
		12.7	2.52	7.7
9.	D	41.4	1.64	16.2
		10.2	3.19	7.0
	P	14.0	1.41	4.1
		23.4	1.55	8.3
	P	17.5[a]	1.48	5.7[a]
		23.6	1.30	5.4
	P	29.0	1.18	4.4
		19.8	1.32	4.8
	P	3.1	1.44	0.9
		6.9	1.12	0.7
	D	17.6	2.93	11.6
		27.2	1.97	13.4
10.	P	12.9	1.36	3.4
		7.5	1.79	3.3
11.	P	39.1	1.23	7.3
		18.5[a]	1.35	4.8[a]
	P	25.0	1.08	1.9
		18.5	1.18	2.8
	P	34.5	1.12	3.7
		20.5	1.21	3.6
		12.6	1.23	2.4
	P	31.7	1.51	10.7
		18.5[a]	2.12	9.8[a]
	P	31.6	1.21	5.5
		21.7	1.23	4.1
		14.4	1.53	5.0
12.	P	24.7	1.10	2.2
		12.1	1.16	1.7
13.	P	23.7	1.01	0.2
		17.8	1.19	2.8
	P	19.2	1.04	0.7
		13.9	1.06	0.8
	P	11.8	2.14	6.3
		15.9	1.65	6.3
	P	22.1	1.10	2.0
		14.2	1.03	0.4
	P	19.2	1.33	4.8
		29.5	1.38	8.1
14.	P	30.9	1.14	3.8
		19.5	1.24	3.8
15.	P	22.4	1.69	9.1
		30.0[a]	1.43	9.0[a]
	P	19.6	1.28	4.3
		26.6	1.12	2.9
	P	31.4	1.25	6.3
		18.3[a]	1.46	5.8[a]
	P	20.4	1.48	6.6
		30.0[a]	1.39	8.4[a]
16.	P	22.5	1.15	2.9
		8.9	1.17	1.3
	D	22.5	1.12	2.4
		14.1	1.39	3.9
17.	P	32.4	1.38	8.9
		15.4	1.57	5.6
	D	18.4	2.50	11.0
		37.9	1.35	9.8
18.	P	14.1	1.42	4.2
		20.4	1.24	3.9
19.	P	23.7	1.25	4.7
		17.4	1.38	4.8
	P	20.0	1.96	9.8
		16.2	2.14	8.6
	P	19.9	3.49	14.2
		28.2	1.86	13.0
	P	30.3	1.10	2.8
		16.4	1.05	0.8
		11.4	1.10	1.0
	D	16.0	7.39	13.8
		25.2	3.05	16.9
20.	P	22.1	1.76	9.5
		16.4	1.92	7.9
	P	22.9	1.11	2.3
		18.7	1.19	3.0
		14.1	1.29	3.2
21.	D	21.1	1.72	8.8
		15.1	1.88	7.1
	D	27.3	1.01	0.3
		22.3	1.19	3.6
	D	12.9	2.09	6.7
		26.4	1.44	8.1
	D	25.4	3.28	17.7
		30.9	1.73	13.0
Mean, standard deviations				
	P	25.0	1.26	4.7
		±6.5	±6.22	±3.1
	$N=50$			
		15.4	1.46	4.3
		±4.2	±0.43	±2.8
			$P<0.01$	$P>0.05$
	D	28.1	1.62	9.4
		±7.4	±0.52	±5.5
	$N=21$			
		15.7	2.38	8.0
		±4.4	±1.37	±4.5
			$P<0.01$	$P>0.05$

Abbreviations: *CF/PF*=ratio of ^{3}H-inulin in collected fluid and perfusate; *C*=total rate of reabsorption of perfusate up to site of collection; P=collection from late proximal tubule; D=collection from early distal tubule.

[a] *In vivo* perfusion rate not measured for this collection and perfusion rate used to calculate *C* was the average *in vivo* rate at the same pump setting. Groups 1–21 represent different animals.

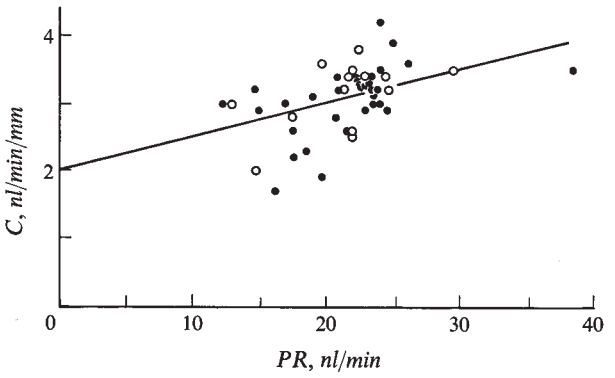

Fig. 6. *Relationship between perfusion rate* (PR) *and reabsorptive rate* (C) *during perfusion with an ultrafiltrate of plasma (open symbols) or with artificial perfusate during the first minute of perfusion (solid symbols).* Each point represents one to twelve measurements in a single animal. The line represents the least squares regression, $C=2.00+0.05\ PR$, $N=44$, $r=0.42$, $F=10.7$, $P<0.01$.

filtrate, and after collections were made from the late proximal or early distal tubule the rate of perfusion either was increased or decreased and re-collections were made from the same tubular site. Details of these experiments are presented in Table 1. The actual rates of perfusion were calculated *in vivo* from the collected perfusate; overall the high rate of perfusion averaged 26.3 ± 7.4 nl/min and the low rate of perfusion averaged 15.8 ± 4.4 nl/min. Reabsorption at the site of collection from proximal tubules averaged 4.7 ± 3.1 nl/min during the higher perfusion rate and 4.3 ± 2.8 nl/min during the lower perfusion rate. Reabsorption at the site of collection from distal tubules averaged 9.4 ± 5.5 at the higher perfusion rate and 8.0 ± 4.5 nl/min at the lower perfusion rate. These values were not statistically different ($P<0.1>0.05$). However, scatter of the data was such that relatively large changes would not have been detected at the lower reabsorptive rates (shorter tubular segments). When only reabsorptive rates greater than 6 nl/min (corresponding to tubular segments of approximately 2 mm) are considered, reabsorption at the site of collection from proximal tubules averaged 8.2 ± 1.9 nl/min during the higher perfusion rate and 6.9 ± 2.7 nl/min during the lower perfusion rate. For distal tubular collections reabsorption during the two rates of perfusion averaged 12.2 ± 3.5 and 9.9 ± 3.7 nl/min, respectively. On paired data analysis these differences in reabsorption at the two perfusion rates were statistically significant for both the proximal and distal collections ($P<0.01$ and 0.05, respectively). Thus, when only these longer tubular segments are considered, a decrease in perfusion rate of 40% was associated with a decrease in reabsorption averaging 17%. In other words, the change in reabsorptive rate averaged 43% of the change in rate of perfusion. In 44 animals the length of perfused segment was measured by microdissection and the absolute reabsorptive rate per unit tubule length was calculated for perfusion rates ranging from 12 to 38 nl/min. The relationship between perfusion rate and rate of reabsorption in these tubules is shown in Fig. 6. Although the scatter of data is great, there is significant ($P<0.01$) positive correlation between reabsorption and perfusion rate ($r=0.42$). The slope of this regression indicates a 20% decrease in reabsorptive rate when perfusion rate is decreased 50%, or a change in reabsorptive rate which is 40% of the change in the rate of perfusion. Because of the scatter of the data, the foregoing analysis should be regarded more in a qualitative than a quantitative sense.

Discussion

The technique of *in situ* microperfusion has been used by several groups of investigators to study factors affecting tubular reabsorption [1–7]. The purpose of the present studies was to examine some potential sources of error which if uncontrolled could influence results obtained with the procedure. Inaccurate estimation of perfusion rate, loss of perfusate, absorption of the volume marker and errors in collection of perfusate downstream all could affect calculated rates of absorption and the conclusions reached from such calculations. Moreover, experimental maneuvers designed to study physiological mechanisms could alter these technical variables and lead to erroneous conclusions regarding the mechanism under study.

The perfusion apparatus used in this study was the same as that described in previous reports from this laboratory [4, 7]. It delivers a constant volume over periods of time in excess of those actually employed in the experiments, and the rate of volume delivery as measured by the DC current output is linearly related to the speed of the motor driving the pump. However, these specifications alone do not provide assurance that the perfusion rate under actual experimental conditions can be assumed from the *in vitro* calibration, since outflow resistance could be different or perfusate could leak out of the tubular lumen through the site of entry of the perfusion pipette. In the present experiments the perfusion rate was measured *in vivo* from the concentration of inulin-methoxy-^{3}H or ^{125}I-iothalamate and the total volume of perfusate collected downstream. Perfusion rates measured *in vivo* averaged 95 ± 10% of the value predicted from the pump speed and directly measured volume delivery *in vitro*.

In some of the present experiments and in previous studies from this laboratory ^{125}I-iothalamate was used as the marker for volume absorption. It has been reported that under some experimental conditions in the rat, iothalamate injected into the proximal tubule is not recovered completely in the urine [12]. In the present studies the recovery of ^{125}I-iothalamate downstream in the proximal tubule averaged 95.6 ± 8.9% when calculated in the same manner as that for inulin. Incomplete recovery of this marker in other studies may relate to the effects of the specific maneuvers employed, viz., ureteral or renal venous obstruction

[12]. We conclude on the basis of our present results that ^{125}I-iothalamate is equal to ^{3}H-inulin as a marker for volume absorption by the proximal tubule, at least under the present experimental circumstances. Also, the recoveries of either ^{125}I-iothalamate or ^{3}H-inulin were not different when a second collection was made from the same tubular site during continuous microperfusion.

When artificial perfusate was used, the rate of proximal tubular reabsorption during the second collection was 34% lower than the rate measured during the first collection approximately ten minutes earlier. An apparent lowering of reabsorptive rate could be due to contamination of the collected sample with interstitial fluid or tubular fluid from adjacent nephrons. This possibility was examined by measuring chemical inulin in the collected perfusate in all experiments in which the absorption marker was ^{125}I-iothalamate. The technique for measuring inulin chemically permitted the detection of at least a 10% contamination of collected fluid with interstitial fluid or plasma. An even smaller contamination with adjacent tubular fluid would have been detected, since inulin would be concentrated above the level present in plasma or interstitial fluid. In the course of the study a few samples of collected perfusate contained inulin and were discarded. The samples accepted contained no inulin; therefore, the lower rate of tubular reabsorption observed during the second collection could not have been due to contamination of perfusate with interstitial fluid or fluid from tubules adjacent to the one being perfused. Also, since the recoveries of markers used for volume absorption were virtually complete during the second collections as compared to the first, and since the rates of perfusion measured *in vivo* were unchanged with time, neither of these two possible sources of error accounted for the lower calculated rate of reabsorption at ten minutes.

When the perfusion solution was a protein-free ultrafiltrate of normal rat plasma, the rate of proximal tubular reabsorption measured twice during continuous perfusion of the same tubular segment remained constant over the ten-minute period of observation. This result indicated that the decreasing rate of reabsorption measured when the artificial perfusate was used must be due to the presence of, or absence of, something in the perfusion solution. Although the perfusate contained glucose and urea in addition to the major plasma electrolytes, it did not contain amino acids, other organic acids and some plasma inorganic ions such as phosphate, sulfate and magnesium. It seems possible that the absence of some such constituent of plasma could result in a gradual leaching of the substance from tubular cells and that this loss might influence the tubular reabsorptive rate. We have made no systematic attempt to determine what this constituent is, but it seems reasonable that the falling rate of reabsorption observed with the artificial solution could be due to the absence of some substance(s), normally present in glomerular filtrate, which influence one or more steps involved in the reabsorption of sodium. For instance, the entrance of sodium into cells by facilitated diffusion appears to be coupled to the facilitated diffusion of certain amino acids [9]. Moreover, Kokko [13] has reported that potential differences across the proximal tubule are influenced by the presence of amino acids in the lumen.

It is important to emphasize that the lower rate of tubular reabsorption during perfusion with the artificial perfusate was evident only during the second collection. Collections begun during the first minute of perfusion yielded reabsorptive rates for plasma ultrafiltrate and the artificial solution which were not statistically different. These observations suggest that measurements made early during microperfusion with an artificial solution may be reliable in a quantitative sense, but those made after longer periods of perfusion may not be. In order to avoid this possible source of error in the future we suggest that tubular perfusions can be performed with an ultrafiltrate of plasma.

If normal plasma contains filterable substances that act on the luminal membrane of proximal tubules to maintain a constant rate of reabsorption, it is tempting to speculate that such substances could be involved in alterations of reabsorptive rate under some experimental conditions such as volume expansion or a reduction in glomerular filtration rate.

The observation of a falling rate of proximal tubular reabsorption when tubules were perfused with the artificial solution raises important questions regarding both the quantitative and qualitative meaning of data obtained from such microperfusion studies. Previous studies employing *in situ* microperfusion of proximal tubules have yielded conflicting results regarding an effect of perfusion rate per se on the rate of reabsorption of sodium. Wiederholt et al [2] reported that within certain limits the rate of reabsorption changed in close proportion to a change in the rate of perfusion. On the other hand, Morgan and Berliner [3], also using *in situ* microperfusion, found no such relationship. Buentig and Earley [4] reported a 20% decrease in the rate of reabsorption when the rate of perfusion of proximal tubules was decreased 50%. Artificial perfusates were used in all of these previous studies, and, in addition, other variables (such as contamination with interstitial or tubular fluid) were not examined.

For the above reasons it seemed important to re-examine the effect of changes in the rate of *in vivo* perfusion on the rate of proximal tubular sodium and water reabsorption using an ultrafiltrate of plasma as the perfusion solution. Two rates of perfusion were examined in the same tubular segment. The high and low rates were selected by setting the pump speed, but the actual rate used to calculate reabsorption was determined *in vivo* by total collection of the non-reabsorbable marker. In re-collection experiments when the perfusion rate was changed approximately two-fold, overall there was no significant change in the rate of reabsorption. However, the actual rate of reabsorption by the total length

of tubule perfused ranged from less than 2 to more than 16 nl/min. This corresponds to segment lengths of less than 1 mm to greater than 4 mm. At the lower rates of reabsorption (shorter segment lengths) large percentage changes in reabsorption could occur but not be detected because of analytical errors such as inaccuracies in measuring perfusion rates and small changes in concentration of the volume marker. When the effect of perfusion rate on reabsorption was analyzed by the re-collection technique, only for experiments in which reabsorption was greater than 6 nl/min (segments longer than approximately 2 mm), the calculated rate of reabsorption was significantly greater at the higher than at the lower rate of perfusion. This same relationship between perfusion rate (using either perfusate) and reabsorptive rate was apparent in unpaired observations in which segment lengths were measured and reabsorption per unit length was calculated for a wide range of perfusion rates. Although the present findings suggest that intraluminal flow rate (load) per se may influence the rate of proximal tubular reabsorption, the scatter of data is great, and statistical significance of flow-dependent reabsorption in paired studies required an arbitrary separation of the data into two groups.

The limitations in measuring relatively large changes in reabsorption over short lengths of tubule, coupled with the fact that previous studies [2–4] utilized artificial perfusates, make it very difficult to reach firm conclusions regarding flow-dependent reabsorption with *in vivo* microperfusion techniques. Burg and Orloff [8] found only a minimal effect of perfusion rate on reabsorptive rate when isolated proximal tubular segments were perfused *in vitro* using an ultrafiltrate of plasma. However, it is possible that tubules *in vitro* are incapable of responding in the same manner as they would at higher reabsorptive rates that may exist *in vivo*. Using this same *in vitro* perfusion technique, Imai and Kokko [14] reported that permeability of proximal tubules to Na and Cl was flow-dependent, a finding consistent with flow-dependent changes in reabsorption rate.

We would not attempt to say on the basis of the present data whether or not intraluminal load can account for a major portion of the balance between filtration rate and proximal tubular reabsorption observed during free-flow collections from late proximal tubules. Different kinds of evidence have been presented both against [3, 5, 6, 8] and in support of [2] this concept. Because of the increasing body of evidence supporting a role of peritubular physical factors in the regulation of proximal tubular reabsorption, attention has been focused recently on the peritubular capillary circulation as the mechanism coupling filtration rate and proximal tubular reabsorption [15–17]. However, the latter hypothesis remains unproved, and to our knowledge a role of intratubular load as a determinant of tubular reabsorption *in vivo* has not been rigorously excluded. *In vivo* microperfusion may not be a suitable experimental technique for resolving the issue, since even if the major analytical hurdles were overcome, it is impossible to perfuse lengths of proximal tubule as long as those studied in usual free-flow experiments.

In the present study when the perfusion pipette was introduced into early or midproximal tubules and collections were made from early distal tubules, resorptive rates equivalent to 5-mm lengths of proximal tubule were obtained. When such segments were perfused at high and low rates, there was a concordant change in the rate of reabsorption which on the average produced 47% balance between load and reabsorption. Such a relationship could be due to changes in reabsorption exclusively by the perfused segment of proximal tubule if minimal water absorption occurs within the loop of Henle [18–21]. However, if passive water loss occurs out of the descending limb of Henle's loop, then a relationship between perfusion rate and volume absorption would be expected. In either case, there appeared to be a direct relationship between perfusion rate and rate of reabsorption by these longer tubular segments which included both proximal tubule and the loop of Henle.

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